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<p>(21) International Application Number: PCT/US84/00540 (22) International Filing Date: 11 April 1984 (11.04.84) (31) Priority Application Number: 483,508 (32) Priority Date: 11 April 1983 (11.04.83) (33) Priority Country: US (60) Parent Application or Grant (63) Related by Continuation US 483,508 (CIP) Filed on 11 April 1983 (11.04.83) (71) Applicant (for all designated States except US): MID-AMERICA DAIRYMEN, INC. [US/US]; P.O. Box 1837, S.S. Station, 800 West Tampa Street, Springfield, MO 65805 (US).</p>		<p>(72) Inventor; and (75) Inventor/Applicant (for US only) : REDDY, Malireddy, S. [US/US]; 1983 Telluride Street, Aurora, CO 80011 (US). (74) Agents: COLLINS, John, M. et al.; Schmidt, Johnson, Hovey & Williams, 1400 Mercantile Bank Tower, 1101 Walnut Street, Kansas City, MO 64106 (US). (81) Designated States: AT (European patent), AU, BE (European patent), BR, CH, CH (European patent), DE, DE (European patent), DK, FI, FR (European patent), GB, GB (European patent), JP, LU (European patent), NL, NL (European patent), NO, SE, SE (European patent), US. Published With international search report.</p>
<p>(54) Title: CHEESE STARTER MEDIA AND METHOD OF MAKING SAME (57) Abstract Low cost, readily dispersible, phage-resistant cheese starter media which include milk-derived nutrients (e.g., nonfat milk and whey) along with a minor proportion of preferably free or unbound lecithin. The media also may advantageously include sodium tetraphosphate which assists in the dispersion of whey solids. The media of the invention can be used at significantly lower levels as compared with nonfat dry milk solids (e.g., 7 percent versus 12 percent), while nevertheless obtaining essentially equivalent results in terms of culture growth and final culture properties. A method of producing the media is also disclosed, involving liquid preblending of phosphates and lecithin, followed by addition thereof to milk-derived nutrients and reaction of the phosphates to tie up free calcium ion. The final step involves drying of the mixture to yield a substantially homogeneous, reconstitutible powder. In other cases the phosphate-lecithin preblend can be dried for later addition to milk-derived nutrients to produce a final starter medium.</p>		

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1 CHEESE STARTER MEDIA AND METHOD OF MAKING SAME

 This is a continuation-in-part of appli-
cation Serial No. 06/483,508, filed April 11, 1983
5 and entitled "Cheese Starter Media."

Background of the Invention

 1. Field of the Invention

 The present invention is broadly con-
10 cerned with novel, low cost cheese starter media
 which can be used by cheese makers in the growth
 of bulk starter cultures, along with a unique
 method of producing the media. More particularly,
 it is concerned with such media which in preferred
15 forms include a minor amount of free or unbound
 lecithin, and/or minor quantities of sodium tetra-
 phosphate for purposes of imparting a stable,
 homogeneous dispersion of the starter media ingre-
 dients; in its method aspects, the invention
20 involves preparation of a liquid preblend includ-
 ing phosphate anti-bacteriophage agent(s) and,
 preferably, lecithin, and mixing of the preblend
 with milk-derived nutrients (e.g., whey and nonfat
 milk), followed by drying to yield a smooth,
25 consistent, substantially uniform and homogeneous
 reconstitutable powder.

 2. Description of the Prior Art

 In the manufacture of natural cheese,
 milk in a cheese vat is inoculated with a minor
30 amount (e.g. 2-4 percent) of a bulk starter pro-
 viding the necessary culture of acid-forming
 microorganisms used for the particular cheese
 being manufactured. For example, in the case of
 Italian cheeses such as mozzarella, it is the
35 usual practice to employ Streptococcus thermo-



1 philus together with one or more lactobacilli such
as Lactobacillus bulgaris. In the art, the strep-
tococci are generally referred to by the short
name of "coccus", while the lactobacilli are
5 referred to as "rod" bacteria because of their
appearance under microscopic examination.

 The quantity and activity of cheese-
making microorganisms can be critical to the
overall outcome of the process and final cheese
10 quality. Again referring to Italian cheese, it
has been found that, in order to make acceptable
cheese, the ratio of coccus to rod microorganisms
in the starters should be from about 1:1 to 5:1,
the most preferable level being about 2:1 to 3:1.
15 If these ratio considerations are not met, the
final Italian cheese product may be deficient in
flavor or physical properties such as elasticity
and "stringiness."

 It is the universal practice among
20 cheese makers to grow their bulk starters using
relatively minor amounts of seed culture. In such
techniques, the seed culture is inoculated into a
starter medium and allowed to incubate therein so
that the culture cells will multiply to produce
25 the desired bulk starter for use in cheese making.
Here again, the types of starter media and the
techniques used during the incubation process can
have a relatively critical outcome on the quality
of the final bulk starter, and hence on the cheese
30 ultimately produced. A dilute dispersion of
nonfat milk (e.g., 12 percent solids level) in
water has long been considered the starter medium
of choice. However, use of nonfat milk in this
context is a relatively expensive proposition, and
35 therefore cheese makers have in the past sought to

1 use media of a less expensive nature which either
eliminate nonfat milk entirely, or sharply limit
its use by provision of substitute materials.

5 For example, U.S. Patent No. 3,852,158
describes a starter media which includes milk-
derived materials, a nitrogen source, and citrate
anion. In preferred forms, the starter media
described in this patent contain a major amount of
sweet whey and a minor amount of nonfat dry milk
10 solids.

U.S. Patent No. 3,998,700 describes
starter media which include both acid and sweet
whey solids together with nonfat dry milk solids.
Finally, U.S. Patent No. 2,805,950 describes the
15 use of whey for culturing bacterial microorganisms
used in making a swiss cheese.

While a number of alternative starter
media have thus been proposed in an attempt to
provide an acceptable substitute for expensive
20 nonfat milk, none of these media have given re-
sults completely equivalent to that of the nonfat
milk. In many cases, the alternative media do not
provide the ideal environment for bacterial
growth, or in the case of Italian cheese making,
25 the final coccus to rod ratio obtained may be
improper. Moreover, in those media which incor-
porate relatively large quantities of whey, a
problem arises by virtue of the phenomenon known
as "whey out." Specifically, large amounts of
whey in a starter medium can precipitate to the
30 bottom of the starter tank and create severe
handling problems. In fact, these problems can
become so severe that some cheese makers simply
refuse to use starter media containing substantial
35 amounts of whey, even if growth characteristics of
such media are satisfactory.



1 U.S. Patent No. 3,041,248 to Hargrove
describes the use of various phosphates for the
control of bacteriophage, which are active against
lactic acid bacteria. Indeed, the many starter
5 media presently available include various phos-
phates for the purpose of combatting bacterio-
phage. In this connection, it is known that the
phosphates react with or tie up the readily avail-
able calcium ion present in the media, and this in
10 turn prevents the bacteriophage from adsorbing
onto the specific starter bacterium. In conven-
tional practice, the phosphates are simply added
directly to the remaining dry ingredients of a
starter medium, followed by appropriate blending
15 and bagging. This conventional dry blending
procedure presents a number of practical problems
in the use of starter medium, however, particular-
ly with respect to the phosphate content thereof.

Specifically, the phosphates tend to be
20 of irregular, grainy appearance and size in a dry
condition, and therefore tend to settle out or
stratify in the dry blended media. When the media
are reconstituted in water, problems are presented
not only from the standpoint of solubility (the
25 conventional media are sometimes difficult to
disperse in water), but more important the phos-
phates present may not completely react with free
calcium ion. In order to ensure the most effec-
tive use of the phosphate anti-bacteriophage
30 agents, it is desirable that the dry medium be
smooth, uniform and substantially homogeneous; and
this is particularly the case when it is borne in
mind that the media may be used with radically
different equipment and cheese-making practices
35 from manufacturer to manufacturer. Non-uniformity

1 inevitably means that in certain portions of the
media the phosphate concentration is too low,
while in other portions it is too high; and both
of these conditions should be avoided.

5 In addition, when a typical dry blended
powder medium is reconstituted in water, it is
desirable to allow sufficient time for the phos-
phate to react with available calcium. Under
normal cheese plant conditions, however, this
10 reaction time should be minimized, and in some
instances time constraints have forced cheese
makers to employ a starter medium which has been
insufficiently reacted; the result of this is that
the problem of bacteriophage may not have been
15 completely eliminated, and this in turn can have
severe consequences in terms of cheese production.

In short, the irregular, non-uniform
nature of many dry blended starter media composi-
tions lead to a number of rather serious problems,
most particularly with respect to the proper
20 utilization of phosphate anti-bacteriophage agents
present therein.

Accordingly, there is a heretofore
unsatisfied need in the art for less expensive,
25 alternative starter media, and a method of produc-
tion thereof, which can be used in lieu of nonfat
milk per se while giving essentially equivalent
results in terms of culture growth and qualities,
and which avoids practical difficulties such as
30 "whey out" and problems stemming from non-
uniformity.

Summary of the Invention

35 The problems outlined above are in large
measure solved by the present invention which



1 provides greatly improved starter media for cheese
making microorganisms. A principal advantage of
the invention is the fact that dried media can be
readily produced which are virtually homogeneous
5 and give the appearance of a fine, talcum-like
powder. The uniformity of the media of the inven-
tion facilitates reconstitution and dispersal
thereof in water or other aqueous media and sub-
stantially prevents differential phosphate concen-
10 trations.

In terms of production methods, dried,
reconstitutable bacteriophage-resistant starter
media for cheese-making microorganisms are pro-
duced by first providing a quantity of milk-
15 derived nutrient such as whey, nonfat dry milk
solids, or a combination of the foregoing. In the
next step, a liquid preblend is prepared, sepa-
rately from the first quantity of milk-derived
nutrients, with the preblend having a phosphate
20 anti-bacteriophage agent dispersed therein.
Advantageously, this preblend also includes a
quantity of lecithin along with appropriate miner-
als. The liquid preblend is then added to the
milk-derived nutrients to form a liquid mixture,
25 and this mixture is then allowed to react for a
period of, typically, 1-12 hours in order to
permit the phosphates to react with available
calcium ion in the mixture. In the final manu-
facturing step, the reacted mixture is dried,
30 usually using conventional spray drying tech-
niques.

In preferred manufacturing procedures,
the pH of the milk-derived nutrients is adjusted
to a level of from about 6.0 to 7.5 prior to the
35 addition of the liquid preblend. Further, the



1 milk-derived nutrients may be partially concen-
trated to a solids level of from about 25 to 50
percent, and the temperature thereof is advan-
tageously adjusted to from about 35 to 60 degrees
5 Fahrenheit, in order to facilitate dispersal of
the preblend therein.

The preblend is normally prepared by
heating a quantity of water to a temperature of
from about 85 to 130 degrees Fahrenheit, followed
10 by addition of the phosphate agent(s) such as mono
and disodium phosphate and sodium tetrphosphate
to the heated water, with agitation to achieve a
substantially uniform dispersion. In preferred
procedures, an amount of free or unbound lecithin
15 is also added to the preblend mixture.

The final dried starter media in accord-
ance with the invention are very uniform and
homogeneous, and exhibit extremely desirable
physical characteristics which greatly facilitate
20 their use. As noted, such media in dried form
broadly comprise a milk-derived nutrient and a
minor amount of unbound lecithin. The milk-
derived nutrients used in the preferred starter
media advantageously comprise relatively modest
25 amounts of nonfat milk, and major proportions of
whey. Starter media in accordance with the in-
vention which include substantial whey fractions
are greatly improved by the addition of a minor
amount of sodium tetrphosphate therein, which as
30 noted is added with the other phosphates in the
liquid preblend. This additive has been found to
greatly assist in the aqueous dispersion of the
whey, and largely eliminates the problem of "whey
out."

35



1 The addition of free or unbound lecithin
to the starter media hereof has been found to give
enhanced results in terms of culture growth and
final bulk starter properties; indeed, the pre-
5 ferred media of the invention give essentially
equivalent results, as compared with use of nonfat
dry milk solids in aqueous dispersion. In fact,
such equivalent results obtain through the use of
significantly smaller quantities of the present
10 media, as compared with NFDM. Specifically, a 7
percent solids dispersion of the preferred media
of the invention gives virtually identical re-
sults, as compared with a 12 percent solids dis-
persion of NFDM.

15 As used herein, the term "free" or
"unbound" in conjunction with lecithin refers to
lecithin in relatively purified form which is
substantially free of chemical and/or physical
bonding to other materials. Such free or unbound
20 lecithin is to be contrasted with, for example,
lecithin which may be found in products such as
fresh milk or buttermilk. In such context, the
relatively minor lecithin content is believed to
exist as a lipoprotein where the lecithin is in a
25 complex with the protein. Such association with
other constituents may have the effect of altering
the properties of the lecithin in the context of
starter media; accordingly, use of the free or
unbound lecithin as herein defined is preferred.

30

35



1 The media of the invention also advantageously include minerals such as manganese chloride and ferrous ammonium sulphate, and a corn steep solids/whey solids stimulant.

5 Although in preferred forms a complete dried starter medium is provided in accordance with the invention, in other instances a dried preblend can be produced for addition to sweet whey or other milk-derived nutrients to produce a
10 complete starter medium. Such a preblend would comprise a dried, substantially uniform powder having therein respective quantities of a phosphate anti-bacteriophage agent, lecithin and optionally NFDM, corn steep stimulant and minerals
15 (e.g., ferrous ammonium sulfate and/or manganese chloride). The lecithin is preferably free or unbound as defined, and is advantageously present at a level of from about 0.1 to 0.8 percent by weight (most preferably, 0.5%).

20

Description of the Preferred Embodiments

 In practice, one of the most preferred starter media of the invention is in the form of a dried composition which can be added to an aqueous
25 system to give a liquid starter medium. This composition includes the following components:

30

35



1

TABLE I

	<u>Ingredient</u>	<u>Initial Quantity</u> ¹	<u>Parts by Wt. of Dried Composition (Dry Basis)</u>
5	Stimulant	700 lbs.	4.120
	Nonfat dry milk solids	900 lbs.	5.290
	Sodium tetra phosphate	800 lbs.	4.700
10	Disodium phosphate	550 lbs.	3.230
	Monosodium phosphate	800 lbs.	4.700
15	Manganese chloride	400 gr.	0.005
	Ferrous ammonium sulfate	400 gr.	0.005
20	Lecithin ²	5 gallons	0.290
	Sweet ³ liquid whey	280,000 lbs.	77.660

25 ¹ Total weight or quantity, including free water,
of starting ingredients

² Lecithin in liquid form, 50% by wt. solids; or
could be in the form of a dried powder

30 ³ May alternatively be derived by mixing 13,198
lbs. of dried whey with 266,802 lbs. of water

35



1 The preferred dried powder media composition is made as follows. In the first step, 700
2 pounds of the stimulant (a dried mixture of corn
3 steep solids and sweet whey solids described in
4 detail below), along with 900 pounds of the nonfat
5 dry milk solids are mixed with the 280,000 pounds
6 of liquid sweet whey (either raw or pasteurized,
7 normally pasteurized). After sufficient mixing to
8 disperse the stimulant and milk solids, the mixture
9 is neutralized by the addition of sodium
10 hydroxide (50%) to a pH of 6.0-7.5 (preferably
11 7.0). The pH adjusted mixture is then thermally
12 evaporated under vacuum conditions to a 25-50
13 percent solids level (preferably 41%), whereupon
14 the mixture is cooled to 35-60 degree Fahrenheit
15 (preferably 50° F.) and transferred to a final
16 mixing tank.

17 A phosphate/minerals/lecithin premix is
18 prepared separately from the mixture of stimulant,
19 dried milk and whey. This premix is made by
20 adding 375 gallons of water at 85-130 degrees
21 Fahrenheit (preferably 96° F.) to a 1,000 gallon
22 mixing tank. Next, 800 pounds of sodium tetra
23 phosphate is added, followed by 800 pounds of
24 monosodium phosphate and 550 pounds of disodium
25 phosphate, all with constant agitation. The next
26 step involves dissolving the 400 grams of ferrous
27 ammonium sulfate and 400 grams of manganese chloride
28 in a small amount of water, whereupon these
29 minerals are added to the agitated mixture of
30 water and phosphates. The 5 gallons of lecithin
31 is then added to the premix tank, again with
32 sufficient agitation to ensure homogeneity. Other
33 phosphate anti-bacteriophage agents can of course
34 be employed in place of the preferred phosphates
35



1 listed above, e.g., ammonium phosphates. More-
over, while dry granular phosphates can be added
and dissolved, in other instances these can be
made in plant by mixing the starting ingredients,
5 e.g., appropriate quantities of phosphoric acid
and ammonia can be reacted to form the desired
ammonium phosphates, and the products used direct-
ly without an intermediate drying step.

10 This premix is then added to the mixture
of whey, stimulant and dried milk solids, where-
upon the overall mixture is agitated for 1-12
hours, and preferably overnight, in order to
assure that the phosphates react with available
calcium ion in the mixture. The reacted mixture
15 is then spray dried to about 3-6 percent moisture
(preferably 4%) to yield a substantially uniform
and homogeneous, flowable, dried, powder-like
material. Of course, if the medium is made in the
plant where it is to be used, it may be advanta-
geous not to dry the medium but rather to use the
20 same directly with appropriate dilution to achieve
the desired solids content for the final liquid
medium, i.e., from about 5 to 15 percent, and more
preferably from about 7-11 percent.

25 In other cases, however, the phosphate/
minerals/lecithin premix can be formulated alone
and dried, whereupon it can later be added to whey
or other milk-derived nutrients to yield a final
medium. This procedure gives some of the advan-
30 tages of the preferred techniques described above,
and avoids the expense of drying, storing and
shipping of large quantities of whey and the other
components of the complete medium.

35 The stimulant referred to above is made
by taking 280,000 pounds of separated raw whey



1 from the cheese-making vat (such amount of whey
being a separate quantity from that used in the
starter media per se listed in Table I), and
5 adjusting the pH thereof to a level of about 8.0
with sodium hydroxide. The pH-adjusted whey is
then evaporated to a 34 percent solids level, and
cooled to 50 degrees Fahrenheit. The evaporated
whey is then pumped into a tank containing 42,880
10 pounds of commercially purchased corn steep liquor
having a pH of 4.15. Such liquor is obtained from
The Staley Corporation of Decatur, Illinois, and
has a 50 percent solids level. This creates a
mixture containing about 60 percent by weight corn
15 steep solids and 40 percent by weight whey solids.
The 60 percent-40 percent mixture is then agitated
overnight, filtered and spray dried to about 4
percent moisture. The resultant dried product is
stored in 50 pound bags for subsequent use in the
20 starter media. An alternate stimulant can be
produced by replacing the corn steep liquor with
liquid yeast extract or any other suitable liquid
stimulant.

25

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1 The above described dried media composi-
tion can be used to obtain starter media for
various types of microorganisms used in cheese
making. For example, in order to obtain a starter
5 media specifically designed for culturing micro-
organisms used in making Italian cheeses such as
mozzarella (referred to as "coccus" and "rod"
microorganisms), the following procedure is fol-
lowed. First, 3,950 pounds (476 gallons) of fresh
10 water is pumped into the starter tank, and the
water is heated to a level of 100-110 degrees
Fahrenheit. Three hundred pounds of the dried
starter medium of Table I above is next added to
the water, with the aid of a powder horn. The pH
15 of this mixture will be about 6.6 ± 0.1 . The
mixture is then heated to 190 degrees Fahrenheit
and held for one hour at this temperature, where-
upon the mixture is cooled to a temperature of
100-112 degrees Fahrenheit. At this point, the
20 mixture is ready for inoculation with the desired
coccus and rod cultures.

 In another example, a dried composition
very similar to that described above can be em-
ployed to prepare a culture medium for lactic
25 cultures to be used in making American-type chee-
ses. From a compositional standpoint, the only
difference involves the addition of a total of
2200 grams of ferrous sulfate. In this technique,
3,950 pounds (476 gallons) of fresh water is
30 pumped into the starter tank, and is heated to a
temperature of 100-130 degrees Fahrenheit. Three
hundred pounds of the modified dried starter
medium is next added to the heated water with the
aid of a powder horn, giving a resultant pH of the
35 mixture of approximately 6.6 ± 0.1 . The mixture

1 is then heated to 190 degrees Fahrenheit, and held
at this temperature for 1 hour, whereupon the
medium is cooled to 74-80 degrees Fahrenheit.
Here again, at this point in the procedure, the
5 medium is ready for inoculation with the appropriate lactic cultures.

While in many instances the preparation of a complete, dried composition of the type described above is advantageous for reasons for ease of handling or the like, the invention is not
10 so limited. That is to say, in appropriate circumstances a cheese manufacturer may wish to use whey derived directly from the cheese making operation, as opposed to having whey in the dried media composition. In such a case, one option
15 would be to prepare a supplement mixture which can be added to liquid whey to produce a final liquid starter media. The stimulant, phosphates, minerals, nonfat dry milk and lecithin are premixed either by dry or wet blending, and are added to
20 liquid whey. The pH of the mixture is then adjusted to a level of 6.6-6.7 through addition of, e.g., sodium hydroxide or ammonia, and the mixture is heated to 190 degrees Fahrenheit and held at
25 that temperature for one hour. The mixture is then cooled to 100-112 degrees Fahrenheit, whereupon it is ready for inoculation with a desired culture.

30

EXAMPLE 1

In this test a comparison was made between the two starter media, namely nonfat dry milk solids, and the most preferred lecithin-
35 containing composition of the invention, in terms



1 of final coccus/rod ratios, bacterial counts and
the activities achieved using the respective
media.

5 The nonfat dry milk was reconstituted in
water at 12.0% solids in water, whereas the medium
hereof (Table I) was reconstituted in water at a
level of only 7 percent solids. Both media were
heat treated by heating to 190 degrees Fahrenheit
and holding at this temperature for one hour. The
10 media were then cooled to 102 degrees Fahrenheit
and inoculated with identical quantities (1%) of
coccus and rod cultures (Streptococcus thermo-
philus and Lactobacillus bulgaris). The cultures
were then incubated in the respective media at 102
15 degrees Fahrenheit until the titratable acidity
exceeded 1.0; for the NFDM system this took about
5.5 hours, and for the medium of the invention
about 7 hours. At this point the cultures were
cooled to 40 degrees Fahrenheit. The two cultures
20 were then tested for titratable acidity, coccus/
rod ratio, pH and activity, all using conventional
techniques. The results of this test were:

TABLE II

Media	Final pH	Final Titratable Activity	Coccus/Rod Ratio	Total Bacterial Count	Activity
NFDM (12%)	4.20	1.02	4:1	140×10^7	0.70
Inven- tion (7%)	4.35	1.02	4:1	130×10^7	0.72

1 As noted in Table II, the results using
the costly NFDM are very similar to those obtained
with the medium of the invention. This is very
surprising in that a significantly greater quantity
5 of NFDM was employed (12%) versus the invention
(7%). At present day typical retail costs, the
cost of using the medium of the invention to
achieve equivalent results is on the order of only
50 percent of that of using NFDM as a starter
10 medium.

In another similar test, equal percent-
age solids amounts of NFDM and the medium of the
invention were tested (7% solids used in both
cases). These tests were conducted in the same
15 manner as heretofore described except that the 7
percent solids NFDM media was incubated after
inoculation until the pH reached about 4.25; this
is the pH level where NFDM systems normally give a
titratable acidity of greater than 1.0. That is
20 to say, if a 7% solids NFDM system is allowed to
incubate to a 1.0% or greater titratable acidity,
the pH would be abnormally low (e.g., around 3.7),
and the bacteria would be injured or the coccus/
rod ratio would be totally unacceptable.

25 Accordingly, the incubation of the 7
percent NFDM system was terminated at the normal
pH achieved for full strength NFDM media, which
took about 5 hours. On the other hand, the medium
of the invention was incubated for a period of
30 about 7 hours until the titratable acidity ex-
ceeded 1.0. The final results were:

35



TABLE III

Media	Final pH	Final Titratable Activity	Coccus/Rod Ratio	Total Bacterial Count	Activity
NFDM (7%)	4.25	0.79	3:1	68×10^7	0.58
Inven- tion (7%)	4.45	1.01	4:1	120×10^7	0.71

Thus, the low solids NFDM system proved deficient in titratable acidity, bacterial count, and activity, as compared with the invention. Moreover, on a cost basis the medium of the invention is far superior, even at equal solids levels.

EXAMPLE 2

In this example, a comparative test was made between a medium in accordance with the invention (Table I) prepared using the preblending and drying procedure of the invention, versus a compositionally identical medium made simply by dry blending all of the ingredients (the lecithin used in this case was also a dried powder). The separate media were then reconstituted in 60 degrees Fahrenheit water, with agitation as necessary to a 7 percent solids level, and cooked at 190 degrees Fahrenheit for one hour. The media were then cooled to 104 degrees Fahrenheit and inoculated with 0.1 ml. of milk grown coccus and rod culture.

The inoculated media were then incubated until the pH thereof dropped to 4.8 (about 5 1/2 hours), whereupon the pH was raised to 6.2 by the



1 addition of 50 percent sodium hydroxide. The
incubations were then allowed to continue until
the titratable acidity in both cases was 1.10.
5 With the liquid preblend, spray dried medium of
the invention, this took a total of about 10 1/2
hours, whereas with the dry blended medium a total
incubation time of 11 1/4 hours was required.

At this point the media were cooled to
55 degrees Fahrenheit and tested as set forth in
10 certain entries of the following Table:

15

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25

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35



1

TABLE IV

COMPARISONS OF LIQUID PREBLENDING AND DRYING vs.
SIMPLE DRY BLENDING ON THE PHYSICAL AND CULTURE
GROWTH-SUPPORTING PROPERTIES OF STARTER MEDIA

5

<u>Comparison Number</u>	<u>Characteristics</u>	<u>Liquid Pre- blend Spray Dried Medium</u>	<u>Dry Blended Medium</u>
1.	Physical Appearance	Smooth and uniform-- similar to talcum powder	Gritty and Irregular sizes of the various in- gredients evident
10			
2.	Stratification of the media	None	Stratified-- Phosphates Tend to settle down in the powder
15			
3.	Solubility	Instantly soluble even in the 60° F. water	5 minutes agitation re- quired to solubilize in the 60° F. water
20			
4.	Initial pH of the powdered medium when reconstituted	6.65	6.30
25			
5.	Precipitation upon reconstitu- tion and heating	None	Consider- visible pre- cipitates settled to bottom
30			
6.	Time to grow coccus and rod culture using the 1 step neu- tralization to arrive at final 1.10 titratable acidity	10 1/2 hrs.	11 1/4 hrs.
35			

	<u>Comparison Number</u>	<u>Characteristics</u>	<u>Liquid Pre- blend Spray Dried Medium</u>	<u>Dry Blended Medium</u>
1	7.	Coccus/rod ratio after growth	3:1	1:1
5	8.	Total bacterial count per gram after culturing	230 x 10 ⁷	200 x 10 ⁷
10	9.	Activity measured in terms of titrat- able acidity	0.65	0.63
	10.	Smoothness of liquid medium after growth of culture	Smooth	Grainy--an appearance of buttermilk

The foregoing Table demonstrates the many advantages obtained through use of the liquid preblend-drying procedure for producing starter media. The smooth, uniform, essentially homogeneous nature of the media of the invention not only facilitates quick, easy handling, but also gives measurably enhanced results in terms of desirable coccus/rod ratios, bacterial counts and activities.

EXAMPLE 3

This example sets forth another preferred media composition in accordance with the invention, which has been formulated to reduce the incubation time needed to reach a final titratable acidity level of about 1.10 from an average of 10 1/2 hours to about 7 1/2-8 1/2 hours.



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TABLE V

Ingredient	Quantity Used
1 Yeast extract	850 lbs.
5 Nonfat dry milk powder	1,000 lbs.
Disodium phosphate	1,479 lbs.
Monosodium phosphate	221 lbs.
Ferrous ammonium sulfate	17 lbs.
2 Manganese chloride	450 grams
Lecithin	2 1/2 gallons
Sweet liquid whey	Balance to yield 17,000 lbs. batch of dried medium

10

1 Employed as a stimulant

2 50% solids liquid lecithin

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The medium of Table IV is made as outlined above in Example 1. In the first step the liquid whey, NFDM powder and yeast extract are mixed and ammonium hydroxide or anhydrous ammonia is added to adjust the pH to 7.0, followed by drying to about 40 percent solids and cooling to 50 degrees Fahrenheit. A liquid preblend of lecithin, the phosphates and the minerals is then made by dispersing these ingredients in 96 degrees Fahrenheit water as outlined in Example 1. The preblend is then added to the cooled nutrients, and the mixture is allowed to react overnight, and the pH is then adjusted as necessary using NH_4OH or anhydrous ammonia. Finally, the mixture is spray dried to about 4% moisture.

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EXAMPLE 4

5 A highly preferred lactic culture medium
for growing cheddar cheese starter microorganisms
is set forth below:

TABLE VI

	<u>Ingredient</u>	<u>Quantity Used</u>
10	¹ Corn steep solids	1,360 lbs.
	¹ Yeast extract	425 lbs.
	Nonfat dry milk powder	1,000 lbs.
	Sodium tetrphosphate	840 lbs.
	Disodium phosphate	435 lbs.
	Monosodium phosphate	1,275 lbs.
	Manganese chloride	250 grams
15	² Ferrous ammonium sulfate	10 lbs.
	Lecithin	2 1/2 gallons
	Sweet liquid whey	Balance to yield a 17,000 lb. batch of dried medium
<hr/>		
20	¹ Stimulants	
	² 50% solids liquid lecithin	

25 This medium is made as outlined above,
wherein the corn steep solids, yeast extract, NFDM
and whey are initially mixed, and a separate
liquid preblend of the phosphates, minerals and
lecithin in water is added thereto, followed by
overnight reaction and drying.

30 The tests described in the foregoing
examples were performed as follows:

35



- 1 pH Hydrogen ion concentration was determined
 using Beckman pH meter

Titratable Acidity

- 5 9 grams of the medium sample was titrated
 with 0.1 N sodium hydroxide using pheno-
 phthalin as an indicator. A faint pink color
 indicated the end point.

Coccus and Rod Ratio

- 10 A one in ten dilution of culture in water was
 smeared on a clean glass slide, stained with
 methylene blue, and examined under a compound
 microscope. The ratio was determined on the
 basis of clump and individual counts.

Total Bacterial Count

- 15 The cultured samples were serially diluted in
 sterile phosphate buffered water according to
 the procedures outlined in the Standard
 Methods for the examination of dairy products
 and plated using tryptic soy agar fortified
20 with 0.5 percent yeast extract. The plated
 samples were incubated at 37 degrees Centi-
 grade for 4 days. The counting and expres-
 sion of the test results were done according
 to the Standard Procedures.

25 Activity Test

- 2 grams of culture was inoculated into 100
 ml. of sterile 10.0 reconstituted nonfat dry
 milk. The nonfat dry milk was pretested for
 the inhibitory compounds. The inoculated
30 milk was incubated at 36 degrees Centigrade
 for 45 minutes. At the end of incubation,
 the temperature was gradually increased to 46
 degrees Centigrade within a span of 30 min-
 utes and it was thereafter maintained at that
35 temperature for a period of 1 hour. The

1 samples were then chilled to prevent any
further acid development. Ten grams of the
sample was carefully weighed into a 25 ml.
5 beaker. Ten drops of indicator (pheno-
phthalein) was added and the entire contents
were titrated against 0.1 N sodium hydroxide
until a faint pink color persisted for 15
seconds. The results were expressed as
percent titratable acidity.

10 Although the above described specific
culture media are preferred in terms of composi-
tion and method of preparation thereof, those
skilled in the art will readily appreciate that
15 the invention is not so limited. That is to say,
in other forms of the invention, various substi-
tute and/or additional materials may be employed,
as opposed to those specifically recited in Tables
I, V and VI, and moreover the amounts of use can
20 be varied for the respective components. To give
but a few examples, the preferred corn steep
solids/sweet whey solids stimulant can be employed
in an amount from about 1-10 percent by weight,
and more preferably from about 2-6 percent by
25 weight (dry basis). Other possible stimulants
useful in this context include yeast extract,
hydrolyzed vegetable proteins, pancreatic enzyme
digests, and protease-treated milk. Such stimu-
lants serve as non-specific growth rate enhancers
30 which increase the rate of acid production and
growth. Stimulants of this type have been known
in the past, particularly in the context of start-
er media which contain other materials besides
strictly nonfat dry milk solids.

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1 While the use of nonfat dry milk solids
and whey is preferred in order to provide the
requisite milk-derived nutrients for the overall
media, other materials such as casein, the various
5 caseinates, casein hydrolyzates, partially de-
mineralized whey, and whey protein concentrates
could also be used. In those instances where
nonfat milk is employed, such should be present at
a level of from about 1 to 10 percent by weight,
10 and more preferably from about 4 to 6 percent by
weight (dry basis). In like manner, when whey is
employed, such should be present at a level of
from about 50 to 90 percent by weight, and more
preferably from about 70 to 80 percent by weight
15 (dry basis).

As noted above, the use of sodium tetra-
phosphate in the media of the invention is pre-
ferred, particularly in those instances where a
major proportion of whey is present. This serves
20 to minimize the extent of so-called "whey-out" by
aiding in the dispersion of the whey solids.
Advantageously, the sodium tetraphosphate is
present at a level of at least about 2 percent by
weight, and more preferably from about 3 to 13
25 percent by weight (dry basis).

The disodium phosphate and monosodium
phosphate additives are employed in the preferred
composition in order to inhibit bacteriophage.
The monosodium phosphate should be used at a level
30 of from about 2 to 8 percent by weight (dry ba-
sis), whereas the disodium phosphate should be
used at a level of about 3 to 13 percent by weight
(dry basis). Moreover, the combination of sodium
tetraphosphate with disodium phosphate and mono-
35 sodium phosphate is particularly preferred inas-



1 much as this combination gives good whey dispersi-
bility, bacteriophage protection, and a buffering
capacity in the overall system.

5 While manganese chloride and ferrous
ammonium sulfate have been employed in minor
amounts as additive minerals, it will be readily
seen that other minerals and levels of use can be
employed. Particular minerals and optimum levels
of use thereof are within the skill of the art.

10 The free or unbound lecithin forming a
part of the preferred media of the invention
should be present at a level of from about 0.05 to
25 percent by weight, and more preferably from
about 0.20 to 1 percent by weight (dry basis).
15 The utility of free or unbound lecithin in the
media of the invention is not fully understood,
but it is believed possible that the presence of
lecithin (a phospholipid) improves the cellular
integrity of the cheese-making microorganisms and
thus promotes growth. In addition, lecithin is
20 known to be an emulsifier, and could assist in the
transport of nutrients into the cellular structure
of the microorganisms. However, it will be appre-
ciated that the foregoing represents hypothesis,
25 and there is no wish to be bound to any sort of
theory of operability in connection with use of
lecithin.

During use of the media in accordance
with the invention, it is desirable that the final
30 liquid medium have a solids content of from about
5 to 15 percent by weight and more preferably from
about 7-12 percent by weight, and most preferably
from about 7-8 percent by weight. Obviously, use
of the smallest amount of solids is preferred for
35 economic reasons.



1 A variety of culture-growing techniques
can be employed with use of the media of the
invention. As noted above, the traditional tech-
5 nique of inoculating the medium with microorgan-
isms at a starting pH in the range of, typically,
6.0-6.5, followed by incubation until the medium
exhibits a pH in the range of 4.0-4.5, gives
excellent results. In addition, certain other pH
10 modification techniques described in recent years
(see, e.g., U.S. Patent No. 4,282,255) can be used
to good effect in conjunction with the improved
media of the invention.

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1 Claims

1. A method of making a dried, re-
constitutable, bacteriophage-resistant starter
medium for cheese-making microorganisms, said
5 method comprising the steps of:

 providing a first quantity of milk-derived
 nutrient;
 preparing, separately from said first quanti-
 ty of milk-derived nutrient, a liquid
10 preblend having a phosphate anti-
 bacteriophage agent dispersed therein;
 adding said liquid preblend to said milk-
 derived nutrient to form a liquid mix-
 ture, and allowing said agent to react
15 with available calcium ion in said
 mixture; and
 drying the reacted mixture.

2. The method of Claim 1, said nutri-
20 ent comprising sweet whey.

3. The method of Claim 1; said nutri-
 ent comprising nonfat milk.

25 4. The method of Claim 1, including
 the step of adjusting the pH of said first quanti-
 ty of milk-derived nutrient to a level of from
 about 6.0 to 7.5.

30 5. The method of Claim 1, including
 the step of drying said first quantity of milk-
 derived nutrient to a level of from about 25 to 50
 percent solids.

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1 6. The method of Claim 5, including
the step of adjusting the temperature of the dried
nutrient to a level of from about 35 to 60° F.

5 7. The method of Claim 1, said pre-
blend preparation step comprising the steps of:
heating a quantity of water to a temperature
of from about 85 to 130° F.; and
10 adding said agent to the heated water, and
agitating the resultant mixture to
substantially uniformly disperse the
agent therein.

15 8. The method of Claim 7, said agent
being selected from the group consisting of mono-
sodium phosphate, disodium phosphate, sodium
tetraphosphate and mixtures thereof.

20 9. The method of Claim 1, including
the step of agitating the admixed preblend and
milk-derived nutrient prior to said drying step.

25 10. The method of Claim 1, including
the step of allowing said agent to react for a
period of from about 1 to 12 hours prior to said
drying step.

30 11. The method of Claim 1, said leci-
thin being present at a level of from about 0.2 to
1.0% by weight in the final dried medium.

35 12. The method of Claim 1, there being
from about 50 to 90% by weight whey solids and
from about 1 to 10% by weight nonfat milk solids
in the final dried medium.



1 13. A dried, reconstitutable, bacterio-
phage-resistant starter medium made by the method
of Claim 1.

5 14. A preblend for addition to a milk-
derived nutrient to produce a starter medium for
cheese-making microorganisms, said preblend com-
prising a dried, substantially uniform powder
10 having therein respective quantities of a phos-
phate anti-bacteriophage agent, lecithin, one or
more stimulants and one or more minerals.

15 15. The preblend of Claim 14, said
agent being selected from the group consisting of
monosodium phosphate, disodium phosphate, sodium
tetraphosphate and mixtures thereof.

20 16. The preblend of Claim 14, said
lecithin being unbound lecithin.

25 17. The preblend of Claim 14, said
lecithin being present at a level of from about
0.1 to 0.8% by weight.

30 18. A starter medium for cheese-making
microorganisms comprising a dried powder including
whey and a minor amount of sodium tetraphosphate
therein for assisting in the aqueous dispersion of
said whey.

35 19. The medium of Claim 18, said sodium
tetraphosphate being present at a level of from
about 3 to 13% by weight on a dry basis.



1 20. The medium of Claim 18, including
respective minor amounts of monosodium phosphate
and disodium phosphate.

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INTERNATIONAL SEARCH REPORT

PCT/US84/00540
International Application No.

I. CLASSIFICATION OF SUBJECT MATTER (If several classification symbols apply, indicate all) ³		
According to International Patent Classification (IPC) or to both National Classification and IPC Int. Cl. ⁸ C12N 1/20; A23C 21/00; 19/00; 21/02; 9/12 U.S. Cl. 435/253; 426/583, 36, 41, 43		
II. FIELDS SEARCHED		
Minimum Documentation Searched ⁴		
Classification System	Classification Symbols	
U.S.	435/253; 426/580, 582, 583, 34, 36, 42, 43	
Documentation Searched other than Minimum Documentation to the Extent that such Documents are Included in the Fields Searched ⁵		
III. DOCUMENTS CONSIDERED TO BE RELEVANT ¹⁴		
Category [*]	Citation of Document, ¹⁵ with Indication, where appropriate, of the relevant passages ¹⁷	Relevant to Claim No. ¹⁸
A, P	US, A, 4,402,986, published 06 September 1983 Sinkoff et al.	1-20
A	US, A, 3,354,049, published 21 November 1967 Christensen, V.W.	1-20
A	US, A, 3,192,124, published 29 June 1965, Kheshgi, S., see Col. 6, lines 23-24.	1-20
A	US, A, 4,115,199, published 19 September 1978, Porubcan et al. see Col. 3, lines 40-50.	1-20
A	US, A, 4,289,788, published 15 September 1981, Cajigas, S.D., See Col. 6, lines 53-60	14,16,17
A	US, A, 4,289,789, published 15 September 1981, Cajigas, S.D., See Col. 6, lines 4-11	14,16,17
<p>[*] Special categories of cited documents: ¹⁶</p> <p>"A" document defining the general state of the art which is not considered to be of particular relevance</p> <p>"E" earlier document but published on or after the international filing date</p> <p>"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)</p> <p>"O" document referring to an oral disclosure, use, exhibition or other means</p> <p>"P" document published prior to the international filing date but later than the priority date claimed</p> <p>"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention</p> <p>"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step</p> <p>"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.</p> <p>"&" document member of the same patent family</p>		
IV. CERTIFICATION		
Date of the Actual Completion of the International Search ¹		Date of Mailing of this International Search Report ²
28 June 1984		02 JUL 1984
International Searching Authority ¹		Signature of Authorized Officer ¹
ISA/US		David M. Naff <i>[Signature]</i>

FURTHER INFORMATION CONTINUED FROM THE SECOND SHEET

A , P	US, A, 4,382,965, published 10 May 1983, Sandine et al.	1-20
A	US, A, 4,282,255, published 04 August 1981, Sandine et al.	1-20
A	US, A, 3,041,248, published 26 June 1962, Hargrove et al.	1-20
A	US, A, 4,020,185, published 26 April 1977, Andersen et al.	1-20

V. ☐ OBSERVATIONS WHERE CERTAIN CLAIMS WERE FOUND UNSEARCHABLE ¹⁰

This international search report has not been established in respect of certain claims under Article 17(2) (a) for the following reasons:

1. ☐ Claim numbers because they relate to subject matter ¹² not required to be searched by this Authority, namely:

2. ☐ Claim numbers because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out ¹³, specifically:

VI. ☐ OBSERVATIONS WHERE UNITY OF INVENTION IS LACKING ¹¹

This International Searching Authority found multiple inventions in this international application as follows:

1. ☐ As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims of the international application.

2. ☐ As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims of the international application for which fees were paid, specifically claims:

3. ☐ No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claim numbers:

4. ☐ As all searchable claims could be searched without effort justifying an additional fee, the International Searching Authority did not invite payment of any additional fee.

Remark on Protest

☐ The additional search fees were accompanied by applicant's protest.

☐ No protest accompanied the payment of additional search fees.

III. DOCUMENTS CONSIDERED TO BE RELEVANT (CONTINUED FROM THE SECOND SHEET)		
Category *	Citation of Document, ¹⁶ with indication, where appropriate, of the relevant passages ¹⁷	Relevant to Claim No ¹⁸
A	US, A, 4,053,642, published 11 October 1977, Hup et al.	1-20
A	US, A, 3,998,700, published 21 December 1976, Reinbold et al.	1-20
A	US, A, 4,372,979, published 08 February 1983, Reinbold et al.	1-20
A	N, Journal of Dairy Science, Vol. 44, issued July-December 1961, Hargrove et al., Phosphate Heat Treatment of Milk To Prevent Bacteriophage Proliferation in Lactic Cultures, pages 1790-1810, see page 1800, 6th paragraph	1-20
A	N, Utah Science, issued Winter 1979, Richardson et al., USU Lactic Culture System, pages 94-99:	1-20